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Challenges facing LAM urine antigen tests for diagnosing HIV-associated tuberculosis

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Tuberculosis (TB) remains a major cause of global morbidity and mortality. TB control has been particularly undermined by the HIV epidemic in sub-Saharan Africa where TB caseload has increased greatly. Moreover, HIV-coinfection is associated with decreased sensitivity of current routine tests for TB such as direct smear microscopy and chest radiography, resulting in lower case detection rates [1]. Hence, there is an urgent need for rapid, low cost diagnostic tests for active TB disease, especially in HIV-co-infected patients.

Antigen detection assays based on lipoarabinomannan (LAM), a major lipopolysaccharide component of the *Mycobacterim tuberculosis* cell wall, have shown promise and have been commercially developed into ELISA and "point-of-care" lateral flow formats [2]. A meta-analysis of studies using commercial urine LAM assays in patients with microbiologically confirmed pulmonary TB, reported that sensitivity ranged from 13% to 93% and specificity ranged from 87% to 99% [2]. Sensitivity was increased in HIV-positive cases and was highest in those with advanced immune suppression [2]. The sensitivity of urine LAM detection is considered insufficient for screening of unselected TB suspects but has clinical utility among HIV-infected TB suspects with low CD4 cell counts [2]. The underlying causes of the variable sensitivity and specificity of urine LAM testing are not adequately understood and remain an obstacle to wider application. In this article, we review and discuss the biological factors associated with detection of LAM in clinical samples

LAM, a component of cell walls of all Mycobacteria and related Actinomyces, was first fully characterized in the 1980's. LAM is a heat stable lipopolysaccharide with a variable chemical structure and a molecular weight of 19±8.5 kilodaltons (kd). It has profound immunomodulatory activities, mediated via several receptors of the immune system involved in host-pathogen interactions [3].

In contrast to the polysaccharide antigens of *Haemophilus influenza* and *Streptococcus pneumonia*, which are also utilized in urine diagnostics and are relatively poorly immunogenic [4], LAM is highly immunogenic [5]. Both LAM antigen and anti-LAM antibodies have been identified during natural mycobacterial infection in a wide variety of body compartments including cerebrospinal fluid, sputum or bronchoalveolar lavage fluid and pleural fluid [6]. The presence of anti-LAM antibody may impact on the measurement of LAM antigen and *vice versa*. Understanding the quantitative and functional relationship between antigen and host antibody response may aid the further development of LAM-based assays.

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Antibody responses to LAM are highly heterogeneous, varying with the site of TB disease, treatment response and geography. The binding avidity of anti-LAM antibodies for LAM antigen also increases with duration of TB disease [7]. Despite wide commercial availability, a WHO expert committee concluded that current TB serological tests including those detecting anti-LAM antibodies, provide "inconsistent and imprecise findings and therefore should not be used for TB diagnosis" [8].

Despite the poor utility of anti-LAM antibodies for TB diagnostic purposes, they may play a role in determining the fate of LAM antigen, a subject, which has been explored in animal studies. LAM antigen was detectable in mouse urine approximately 17 hours after intra-peritoneal injection of a crude cell wall extract of *M. tuberculosis* [9]. This finding has provided the major rationale for the development of the urine LAM test as a correlate of pulmonary TB. However, in another mouse study, intravenously administered purified LAM was rapidly cleared from the systemic circulation with subsequent localization in the spleen, with no LAM detectable in the kidney [10]. Additionally, the potential effects of circulating anti-LAM antibodies were demonstrated, when mice were pretreated with anti-LAM IgM antibody, which resulted in injected LAM rapidly localizing the liver, with subsequent excretion via the biliary tract [10]. These latter observations of tissue localization of free and immune-complexed LAM are consistent with the known roles of the marginal zone of the spleen to trap particulate antigen and of liver Kupffer cells for the capture of circulating immune complexes. The fate of LAM has not been studied in humans. However, LAM antigen has been shown to circulate systemically in the form of immune complexes [11]. Consequently serum LAM antigen detection assays have incorporated immune complex dissociation to permit detection.

The state of circulating LAM may therefore have major implications for urine LAM antigen detection assays. Free non-antibody associated LAM is of a size comparable to myoglobin (17 kd), which rapidly crosses the glomerular basement membrane. Glomerular filtration of systemically circulating LAM has been the premise to date, on which urine LAM has been interpreted as a correlate of pulmonary TB. However, LAM antigen complexed with IgG (150 kd), IgA (370 kd) or IgM (1000 kd) antibodies would be too large to pass through the normal healthy human glomerulus [12]. Therefore in the presence of circulating anti-LAM immunoglobulin, LAM detected in urine might be more likely to reflect local renal involvement with TB rather than distant pulmonary disease. Supportive evidence for a direct local renal source of LAM was provided by the detection of mycobacteriuria, measured by urine Xpert MTB/RIF assay, in approximately half of urine LAM-positive patients and none of urine LAM-negative patients with HIV and confirmed pulmonary TB co-infection [13, 14]. Since Xpert MTB/RIF detects whole *M. tuberculosis* bacilli, positive results indicate the presence of *M. tuberculosis* organisms in the renal tract [15].

The reported specificity of urine LAM testing from 87% to 99% compared to a "gold standard" of sputum culture most likely relates to the ability to obtain good respiratory specimens and the capability study laboratories to isolate and successfully culture *M. tuberculosis*. Apparent false-positives could result from inclusion of patients with unrecognized subclinical disease and true false-positives from contamination of urine with fungi or non-tuberculous mycobacteria in sufficient quantity to cross-react with the LAM test. However, the proportion of urine LAM derived from renal or pulmonary disease would probably not impact greatly on test specificity, as extra-pulmonary disease in HIV infection is most frequently associated with pulmonary TB.

The reported sensitivity of urine LAM detection for diagnosing pulmonary TB ranges from 13% to 93%, which highlights the major shortcoming of the current urine assay [2]. Sensitivity may be impacted by several mechanisms including the characteristics of the test-

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capture antibody, variable concentration of the urine sample, patient selection, humoral immune response and the proportion of urine LAM derived from either renal or extra-renal TB sources. Tests that incorporate polyclonal antibodies for LAM antigen capture are more likely to recognize the multiple antigenic epitopes of LAM compared with monoclonal antibodies targeted at a single epitope [16]. However, polyclonal antibodies increase the risk of "batch to batch" variation during the manufacturing process [17]. In contrast to limited impact on specificity, the proportion of urine LAM derived from either renal or pulmonary disease will markedly impact on test sensitivity, as pulmonary TB disease occurs most frequently without associated extra-pulmonary TB.

The increasing sensitivity of urine LAM testing with progressive HIV immune suppression (as reflected by falling CD4 cell counts) is the major distinguishing feature from other TB diagnostics such as direct sputum smear microscopy and chest radiography which lose sensitivity with worsening immune suppression [18]. It has been proposed that increased urine LAM might reflect an increased total mycobacterial burden that occurs with progressive immune suppression [19]. In addition, advanced HIV may be associated with the inability to produce high avidity immunoglobulin such that free circulating LAM can be renally filtered into urine. An alternative hypothesis is that *M. tuberculosis* organisms are less able to be anatomically compartmentalized at low CD4 cell counts, leading to increased risk of disease dissemination, resulting in consequent renal involvement.

Tests that detect pathogen-derived antigens are considered to better reflect pathogen burden rather than antibody tests, which measure the host immunological response to the pathogen. However, we present data to support a hypothesis that urinary excretion of LAM will likely be impacted by the humoral immune response. *M. tuberculosis* may either produce urinary LAM within the renal tract or from other sites of TB disease dependent on whether LAM is present in the systemic circulation as free antigen or within an immune complex.

The urine LAM test has been developed into a relatively low cost, rapid, lateral flow test suitable for "point-of-care" testing [20]. The sensitivity and specificity are clinically useful for diagnostic screening of TB suspects with advanced HIV immune suppression in whom TB is likely poorly localized and humoral responses impaired. Future development of LAM testing will require a better understanding of the underlying mechanisms of LAM release from *M. tuberculosis* organisms and the fate of LAM within the human body. Remaining questions include whether LAM is released from live replicating or dying mycobacteria, the role of LAM testing as a measure of response to TB chemotherapy and the interaction between the humoral immune response and LAM test performance.

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